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The Detection of Intrathecal Synthesis of Anti-Herpes Simplex IgG Antibodies: Comparison Between an Antigen-Mediated Immunoblotting Technique and Antibody Index Calculations

Philippe Monteyne,[1] Frauke Albert,[2] Benedikt Weissbrich,[2] Elisabetta Zardini,[3] Maria Ciardi,[4] Graham M. Cleator,[5] Christian J.M. Sindic,[1]* and The European Union Concerted Action on Virus Meningitis and Encephalitis

[1]*Laboratory of Neurochemistry; Catholic University of Louvain, Brussels, Belgium*
[2]*Institute for Virology and Immunology, Universitat Wurzburg, Germany*
[3]*Laboratory of Neuroimmunology, IRCCS, Neurological Institute "C. Mondino", University of Pavia, Italy*
[4]*Department of Infectious and Tropical Diseases, University "La Sapienza", Roma, Italy*
[5]*University Department of Pathological Sciences, Manchester Royal Infirmary, Manchester, United Kingdom*

The detection of intrathecal antibody synthesis was compared by the calculation of antibody indices (AI) derived from ELISA techniques with the detection of virus-specific oligoclonal IgGs by an antigen-mediated capillary blot technique. Twenty-seven paired serum and cerebrospinal fluid (CSF) samples were examined from 15 immunocompetent patients with herpes simplex virus encephalitis (HSE) diagnosed by PCR on early CSF samples. These techniques were also applied to paired samples from 20 multiple sclerosis (MS) patients, 10 patients with other inflammatory neurological diseases and 10 patients with non inflammatory neurological disorders. There was a good correlation between the results obtained by AI and those obtained by immunoblotting, especially in HSE (2 discordant results out of 27). Discrepancies were more frequent (25%) in MS patients where a ''polyspecific'' reaction characterized by low affinity antibodies is known to occur. Some of the discrepancies could, in part, be due to serological cross-reaction with varicella zoster virus. ***J. Med. Virol. 53:324–331, 1997.***

KEY WORDS: intrathecal immunity; herpes encephalitis; cerebrospinal fluid; oligoclonal antibodies; antibody indices

INTRODUCTION

Several studies have shown that detection by polymerase chain reaction (PCR) of herpes simplex virus (HSV) type 1 or type 2 DNA in cerebrospinal fluid (CSF) can be used routinely to provide a diagnosis of HSV encephalitis (HSE). Moreover, the combination of this technique with the detection, in a later stage of the disease, of specific intrathecal antibody production to HSV represents currently the most reliable strategy for diagnosis and monitoring of the treatment of these patients [reviewed by Cinque et al., 1996]. Different techniques can demonstrate a specific intrathecal humoral response of which enzyme immunoassay and the immunoblotting technique are probably the most commonly used. Firstly, antibodies can be detected using sensitive ELISAs [Felgenhauer et al., 1982] in combination with the calculation of a quotient between the levels of antibodies detected simultaneously in serum and CSF. Instead of titers, antibody concentrations can be estimated using optical densities or arbitrary units derived from a standard curve [Reiber and Lange, 1991; Felgenhauer and Reiber, 1992]. Different formulae have been described to calculate this antibody index (AI) [Ukkonen et al., 1981; Klapper et al., 1981; Stiernstedt et al., 1985; Reiber, 1994]. These formulae take into account possible damage to the blood-CSF barrier (BCB). Reiber's formula was also designed to take into account local polyclonal IgG production [Reiber and Lange, 1991]. The second major approach is the detection of specific oligoclonal antibodies by immunoblotting. A highly sensitive antigen-mediated capillary blot technique has been developed to detect HSV-specific IgGs in CSF [Dörries et al., 1984; Boucquey et al., 1990]. It has been suggested that immunoblotting, when used in combination with PCR, provides the most

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*Correspondence to: Prof. C.J.M. Sindic, Laboratory of Neurochemistry, Avenue Mounier 53, B-1200 Brussels, Belgium. E-mail: Sindic@nchm.ucl.ac.be

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accurate laboratory approach for the diagnosis of HSE [Pohl-Koppe et al., 1992], provided that samples collected after onset of antibody synthesis are examined.

Demonstration of intrathecal antibody production is in most instances specific for an ongoing infection of the central nervous system (CNS). However, using enzyme immunoassays as well as immunoblotting techniques, 'polyspecific' reactions against neurotropic viruses including HSV have been demonstrated in multiple sclerosis (MS) [Reiber and Lange, 1991; Felgenhauer and Reiber, 1992] and inflammatory or infectious diseases of the CNS [Sindic et al., 1994].

The aim of the present study was to compare systematically the calculation of an anti-HSV AI derived from an ELISA technique with the detection of intrathecal oligoclonal antibodies. This was undertaken in HSE patients and in patients with MS, other inflammatory neurological diseases (OIND) and non-inflammatory neurological disorders (NIND). Reiber's formula [Reiber, 1994] was used as a standard ratio method. Three other formulae have also been evaluated [Ukkonen et al., 1981; Klapper et al., 1981; Stiernstedt et al., 1985].

MATERIALS AND METHODS

Patients

Four patient groups were studied (n = 55). Group 1 (n = 15; 27 serum and CSF paired samples) consisted of herpes simplex encephalitis (HSE) patients. In all cases, the diagnosis was supported by the detection of HSV DNA in the CSF. Seven patients were followed longitudinally with more than one sample being examined for antibody production. Group 2 (n = 20) consisted of patients with MS. All displayed oligoclonal IgG bands restricted to the CSF. A definite clinical diagnosis had been established in all cases [Poser et al., 1983]. Group 3 (n = 10) consisted of other inflammatory neurological diseases (OIND). One patient suffered from varicella zoster virus (VZV) meningitis, five from aseptic meningitis, one from tuberculous meningitis, and three from Guillain-Barré syndrome. Group 4 (n = 10) consisted of cases of non-inflammatory neurological disorders (NIND). These included three cases of Alzheimer's disease, three patients suffering from amyotrophic lateral sclerosis, two from stroke, one from myelopathy due to cervical arthrosis, and one case from sciatica. Paired CSF and serum samples were stored at −20°C for ELISA and immunoblotting.

PCR

Two PCR procedures were applied to samples from the patients of group 1: either a nested PCR for the detection of both HSV 1 and HSV 2 DNA adapted from Aurelius [Aurelius et al., 1991; Aurelius et al., 1993; Monteyne et al., 1996], or a nested procedure using primers common to both HSV type 1 and 2 followed by a restriction enzyme analysis to differentiate between the two types [Dahm et al., 1990]. These techniques allowed a diagnosis of encephalitis caused by HSV type 1 to be made in all but one case in group I, and type 2 in the remaining case.

Determination of IgG and Albumin Content in Serum and CSF

Albumin and IgG in serum and CSF were assayed by established immunochemical methods (either with Turbiditimer, Behring, Marburg, Germany, or with Beckman nephelometer, Brea, CA).

Immunoaffinity-Mediated Capillary Blotting

Detection of serum and CSF anti-HSV and anti-VZV antibodies was carried out by immunoaffinity blotting [Boucquey et al., 1990; Sindic et al. 1994]. CSF and sera diluted to the same IgG concentration were analysed by isoelectric focusing using agarose gels. Ten μl of paired samples were focused for 70 min at 10°C in a LKB Multiphor Unit. Gels were blotted onto a polyvinylidene difluoride (PVDF) sheet (Immobilon, Millipore, Bedford, MA) coated with a tenfold dilution of either HSV or VZV antigen, or with control antigens (from Whittaker Bioproducts, Walkersville, MD) in Tris Buffered Saline (TBS). Immunoaffinity-mediated capillary blotting was carried out under a uniform weight of 1 kg for 40 min at 10°C. Immunoblots were washed in TBS, dipped into a 0.25% glutaraldehyde buffer (Merck, Darmstadt, Germany), and incubated with a thousandfold dilution of alkaline phosphatase-conjugate rabbit anti-human IgG antiserum (Bio Rad) in TBS containing 0.3% defatted milk powder. The immunoblots were stained using an alkaline phosphatase conjugate substrate kit (from Biorad, Hercules, CA).

The presence of two or more anti-HSV oligoclonal IgG bands was estimated semi-quantitatively by comparing the reactivity pattern in CSF and serum. In the case of a very strong pattern in both CSF and serum, samples were diluted further and retested. A mirror pattern indicating passive transudation of antibodies through the blood-CSF barrier (Andersson et al., 1994) was defined as the presence of oligoclonal IgG antibodies in both CSF and serum samples at the same pH and with the same staining intensity. The results were evaluated blindly and independently by three of us (P.M., E.Z., C.S.). Immunoblots undertaken with control antigens were uniformly negative.

Demonstration of HSV-Specific Antibodies by ELISA

HSV-specific IgG antibodies in serum and CSF were determined by ELISA using a commercially available kit (Enzygnost HSV-IgG, Behring, Marburg, Germany). Briefly, microtiter wells coated with antigen and control antigen were incubated with paired serum and CSF samples using at least two different dilutions each for 3 hr at room temperature. The starting dilutions were 1/1386 and 1/8316 for serum, 1/6 and 1/36 for CSF. After 4 washing cycles, HRP-conjugated anti-

human-IgG was added and incubated for 1.5 hr at room temperature. Following a further 4 washings, TMB substrate solution was added and incubated at room temperature for 30 min. The reaction was stopped by the addition of 0.5 M H_2SO_4 and OD was read with a photometer at 450 nm. A standard curve consisting of eight standards S1 to S8 was included in each run. For S1, a commercially available human serum pool (Kontrollogen LU, Behring, Marburg, Germany) was diluted to give a maximum OD of approximately 2.5 and was assigned a value of 100 arbitrary units. Standards S2 to S8 were obtained by a twofold serial dilution of S1. The standard curve was fairly linear over the range from 0.2 to 2.0 OD Units. Arbitrary units (E) of the different serum and CSF dilutions were calculated by reference to the standard curve and multiplied by the dilution factor to give HSV-IgG_{Ser} and HSV-IgG_{CSF} respectively.

Determination of Antibody Indices

The AI was calculated using the formula described by Reiber [Reiber, 1994]:

$$AI = \frac{E_{CSF}}{E_{serum}} : Q_{IgG} \quad (Q_{IgG} = \text{CSF IgG/serum IgG})$$

For every CSF/serum pair, the maximum IgG ratio that can be expected assuming no intrathecal IgG synthesis was calculated from the albumin ratio (QAlb = CSF Albumin/serum Albumin):

$$Q_{Lim} = 0.93 \sqrt{(Q^2Alb + 6.10^{-6})} - 1.7 \times 10^{-3}.$$

In the case of local synthesis of polyclonal IgG in the CNS, (Q_{IgG} higher than Q_{Lim}) Q_{IgG} was replaced by Q_{Lim} in the formula:

$$AI = \frac{E_{CSF}}{E_{serum}} : Q_{Lim}$$

where Q_{Lim} represents the IgG fraction in CSF originating only from blood.

Q_{Lim} can also be estimated using Reiber's quotient diagrams [Reiber and Lange, 1991]. An AI $\geqslant$ 1.5 was considered indicative of intrathecal antibody synthesis.

Three other formulae have been applied:

$$AI_{QIgG} = \frac{\text{HSV antibody}_{CSF}}{\text{HSV antibody}_{serum}} : Q_{IgG}$$

[Ukkonen et al., 1981]

where intrathecal antibody synthesis was assumed for AI $\geqslant$ 2.

TABLE I. Detection of Intrathecal HSV IgG Synthesis

Patients	HSV-specific oligoclonal antibodies	Antibody index $\geqslant$1.5*
HSE	14/15	14/15
MS	4/20	3/20
OIND	3/10	1/10
NIND	1/10	1/10

HSE = herpes simplex encephalitis; MS = multiple sclerosis; OIND = other inflammatory neurological diseases; NIND = non-inflammatory neurological disorders.
*According to Reiber and Lange, 1991; Reiber, 1994.

The ratio QIgG may also be replaced by the ratio QAlb:

$$AI_{QAlb} = \frac{\text{HSV antibody}_{CSF}}{\text{HSV antibody}_{serum}} : Q_{Alb}$$ [Klapper et al., 1981]

where intrathecal antibody synthesis was assumed for AI $\geqslant$ 1.9.

The ratios of optical densities have also been used, instead of ELISA titers:

$$AI_{OD} = \frac{OD_{CSF} \times DF_{CSF}}{OD_{serum} \times DF_{serum}} : Q_{Alb}$$

(DF = dilution factor) [Stiernstedt et al., 1985]

where intrathecal antibody synthesis was assumed for AI $\geqslant$ 2.

RESULTS

Patients with Herpes Simplex Encephalitis (Group 1, n = 15)

The use of immunoblotting and AI calculation gave similar results. Intrathecal synthesis of HSV-specific antibodies was detected by both techniques in 14 out of 15 immunocompetent patients suffering from HSE (Table I). Twenty-seven samples from these patients were analyzed by both methods (Table II).

The discrepant patient, for whom a diagnosis was not confirmed by either of the techniques used, was the only encephalitis case in the study that was found to be due to HSV type 2, as demonstrated by PCR amplification on an earlier sample (patient 5 in Table II).

A longitudinal study was carried out in 6 cases (patients 9, and 11 to 15 in Table II). This demonstrated a good correlation between the detection of HSV-specific oligoclonal antibodies and the AI as calculated with Reiber's formula. Figure 1 shows a representative example of this data (corresponding to patient 14 in Table II). In this case, no intrathecal synthesis of antibodies was detected by either technique at day 2 after onset while both techniques were positive at day 9 and day 22. The progression of the calculated AI (positive when $\geqslant$ 1.5) is shown (Fig. 1b).

In one sample taken three days after onset of the disease (patient 12 in Table II), specific oligoclonal antibodies were detected while the AI remained normal (Fig. 2a). This AI was negative with the four different

TABLE II. HSE Patients

Patient	Age (years)	Sex	Delay (days after onset)	Immunoblot CSF/serum[a]	A.I.[b] (positive when ⩾1.5)
1	35	M	60	++/+	10.4
2	63	M	14	+++/+	46.5
3	36	M	23	+++/++	71.5
4	9	F	27	+++/++	13.0
5	69	F	14	+/+	1.2
6	65	F	18	++/−	31.0
7	70	M	17	++/+	114.0
8	60	M	48	+++/++	21.0
9	27	F	5	+++/++	2.9
			10	+++/++	33.1
			15	++/+	17.1
10	70	M	5 years	++/+	14.5
11	15	F	5	+/−	4.5
			2 months	++/+	40.8
			3 years	+/−	38.8
12	57	M	3	+/−	1.2
			13	+++/++	17.6
			33	+++/++	6.6
13	34	M	11	+/−	1.5
			25	+++/++	6.0
14	57	M	2	−/−	0.9
			9	++/+	5.6
			22	+++/++	28.2
15	19	M	5	++/++	2.1
			28	+++/++	4.8
			50	+++/+	12.7
			3 months	+++/++	35.1

CSF = cerebrospinal fluid.
[a]The presence of anti-HSV oligoclonal antibodies is semi-quantitatively represented by +, ++, or +++. A pattern noted ++/+ means a pattern with stronger reactivity in CSF than in serum (analyzed at the same IgG concentrations), or with additional oligoclonal antibodies that are restricted to the CSF. Note that no distinction was made between cases with oligoclonal antibodies present only in CSF and those with stronger or additional reactivity in CSF: in such cases, an intrathecal synthesis was considered to occur.
[b]According to Reiber, 1994.

formulae under study (not shown). In contrast, one early sample had a positive AI while a mirror pattern without specific CSF bands was observed by immunoblotting (patient 15 at day 5, Table II).

There was an excellent correlation between the results obtained with the four AI formulae for all the samples from HSE patients. Only one sample was falsely negative. This was determined using Ukkonen's formula (data not shown).

Some patients with HSE were characterized by the detection in immunoblotting of intrathecal production of both anti-HSV and anti-VZV antibodies. This may have been a consequence of the known serological cross-reactivity between these viruses (Ndumbe and Levinsky, 1985; Vandvik et al., 1985) (Fig. 2a).

Patients with Multiple Sclerosis (Group 2, n = 20)

The intrathecal synthesis of virus-specific oligoclonal IgG in MS patients has been re-assessed recently [Sindic et al., 1994]. In the present study, a comparison was made between the detection of specific oligoclonal antibodies with the results obtained by the four formulae used to calculate the AI. Specific oligoclonal antibodies were detected in four patients out of the 20 under study (Table III). The different formulae utilized to calculate the AI appeared to be less sensitive than immunoblotting with 1, 2, or 3 positive cases depending on the formula used. Only patient MS 15 was found to be positive by all the techniques, while discrepancies appeared in five other cases. Two cases were positive by one formula, but were negative by immunoblotting (patients MS 2 and 6). In these two cases, immunoblotting revealed a mirror pattern with faint bands present in both serum and CSF (Fig. 2, patient MS 2). In some MS patients, an intrathecal production of both anti-HSV and anti-VZV antibodies was observed (Fig. 2). For example, in MS patient 1 (Fig. 2 and Table III), a faint anti-HSV and a stronger anti-VZV reactivity were observed while the results of the formulae were negative.

Patients with Other Inflammatory Neurological Diseases (Group 3, n = 10)

Intrathecal HSV antibody synthesis was detected in three patients with other inflammatory neurological diseases by immunoblotting, and in one case by AI calculation (Table I). The latter was a case of aseptic meningitis and was positive by both methods. The immunoblot was positive in two additional cases, one Guillain-Barré syndrome and one zoster meningitis with, in the latter, a strong reaction against VZV (Fig. 2c). These cases, in which intrathecal production of antibodies was detected, were negative by PCR amplification of the HSV genome.

Patients with Non-Inflammatory Neurological Disorders (Group 4, n = 10)

Both Reiber's AI and the immunoblotting technique were positive in one case from this group (Table I). The clinical diagnosis in this case was a sylvian stroke with temporal involvement. However, none of the other formulae used to calculate the AI detected an intrathecal antibody synthesis in this case and the PCR amplification of the HSV genome was unsuccessful. All other samples of the non-inflammatory neurological disorders group were negative by all techniques.

DISCUSSION

Both immunoblotting and ELISA detection of intrathecal antibody synthesis appear to have been sufficiently sensitive to confirm a diagnosis of HSE. Overall, there was a good correlation between the techniques, since an intrathecal synthesis of HSV-specific antibodies was detected by both methods in 14 out of 15 immunocompetent patients with HSE. When considering all 27 samples from these 15 patients, the correlation remained excellent. In one case, immunoblotting was positive as early as day three after onset while the AI was normal at that time (patient 12 in Table II). In contrast, an early sample was positive by AI calculation while a mirror pattern, without CSF-restricted an-

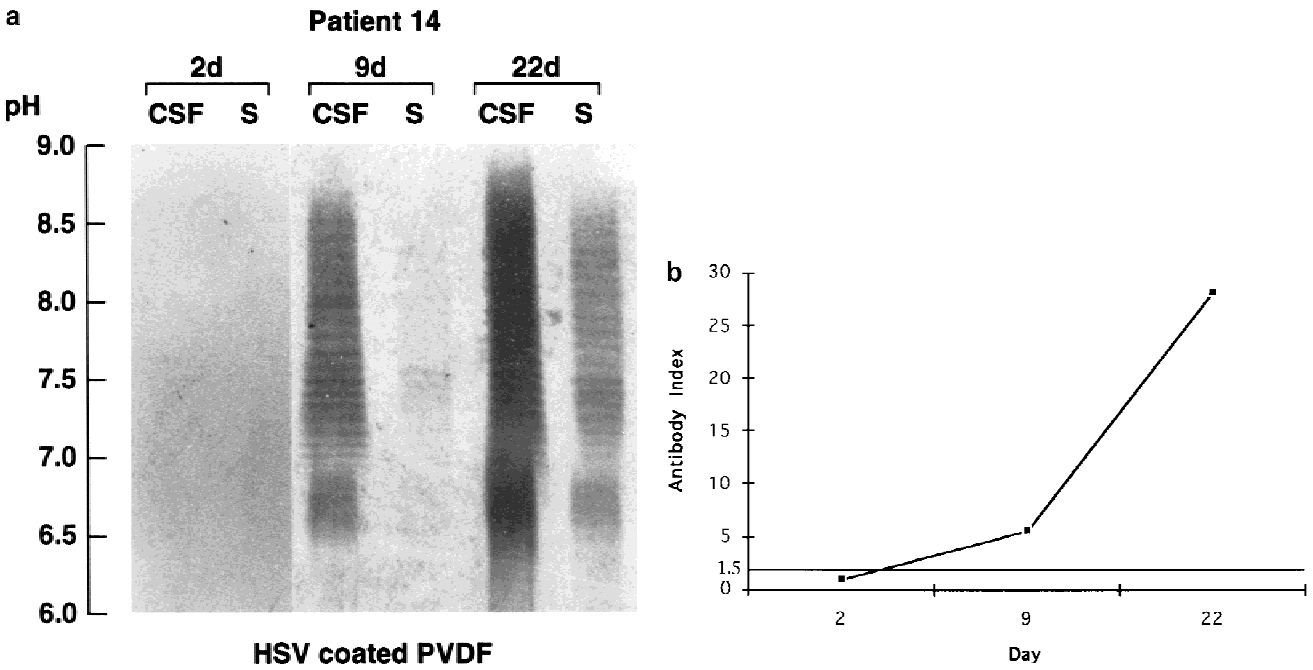

Fig. 1. **(a)** Immunoaffinity-mediated capillary blotting of anti-HSV antibodies, in CSF and serum (S) tested at the same IgG concentration, in the 3 successive samples from patient n°14 with HSE. Patterns are interpreted as follows: 2 days (2d): −/−; 9d: ++/+; 22d: +++/+; **(b)** antibody index as determined according to the formula described by Reiber (1991), in the three successive samples from patient n°14 with HSE.

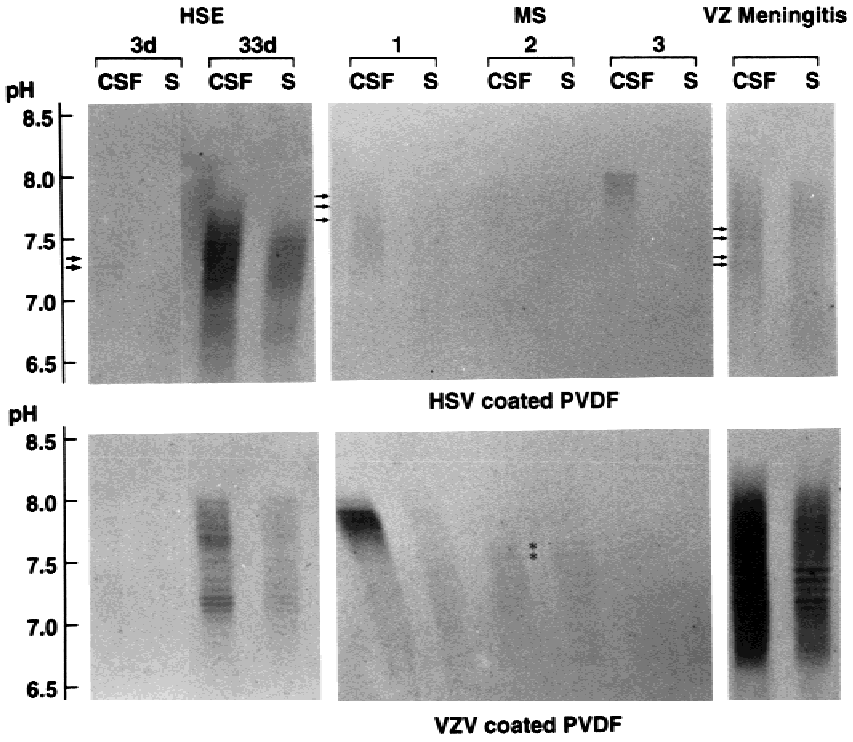

Fig. 2. Representative immunoaffinity-mediated capillary blotting of anti-HSV antibodies (upper part) and anti-VZV antibodies (lower part), in CSF and serum (S) tested at the same IgG concentration, in five patients. **(a)** Patient 12 (see Table II) with herpes simplex encephalitis (HSE); at day 3 (3d), two discrete CSF-specific anti-HSV oligoclonal antibodies (arrows) are detectable in spite of a normal AI (1.2). At day 33, a strong reactivity is present in the CSF and, at a lesser extent, in the corresponding serum (AI = 6.6). A cross-reactivity with VZV antigens is clearly detectable at that time (lower part). **(b)** Immunoblots from three MS patients (1, 2 and 3 from Table III) with HSV AI of 1.0, 0.7 and 3.3 respectively. MS 1 displays faint CSF-restricted anti-HSV oligoclonal antibodies (arrows) and a marked anti-VZV reactivity; MS 2 shows no detectable reactivity on the HSV immunoblot and a mirror pattern of two faint bands reacting with VZV (asterisks); MS 3 is characterized by the intrathecal synthesis of HSV oligoclonal antibodies without VZV reactivity or cross-reactivity. **(c)** In this case of varicella zoster (VZ) meningitis, a very strong anti-VZV reactivity masking oligoclonal banding is observed in the CSF, whereas the immunostaining is less intense in the serum. Faint anti-HSV oligoclonal antibodies present in the CSF (arrows) are likely due to a cross-reactivity (HSV AI = 0.8).

TABLE III. MS Patients: Detection of Intrathecal Synthesis of Anti-HSV Antibodies by Immunoblotting, and by ELISA with Antibody Indices Calculated According to Four Different Formulae

	HSV-specific oligoclonal antibodies[a]	Reiber 1994[b]	Stiernstedt 1985[c]	Klapper 1981[d]	Ukkonen 1981[e]
1	+	−	−	−	−
2	−	−	+	−	−
3	+	+	−	−	+
4 and 5	−	−	−	−	−
6	−	+	−	−	−
7 to 14	−	−	−	−	−
15	+	+	+	+	+
16	+	−	−	−	−
17 to 20	−	−	−	−	−
	4/20	3/20	2/20	1/20	2/20

[a]Positive if only present in CSF or if stronger reactivity in CSF than in serum.
[b]Positive when ⩾ 1.5.
[c]Positive when ⩾ 2.
[d]Positive when ⩾ 1.9.
[e]Positive when ⩾ 2.

tibodies, was observed by immunoblotting (patient 15 in Table II). It is known that HSV DNA is usually detectable in CSF at the onset of neurological symptoms and remains detectable for at least 5 days after initiation of anti-viral treatment, whilst an intrathecal antibody response is usually detectable seven to ten days after onset of the symptoms (van Loon et al., 1989; Aurelius et al., 1991; reviewed in Cinque et al., 1996). In the present study, we confirmed that specific oligoclonal antibodies and increased AI can both be detected as early as 3 to 5 days after onset. It should however be noted that "days after onset" was defined in relation to the time when neurological symptoms were first apparent and thus ignored pre-clinical stages of disease. In atypical cases determination of the time of onset of neurological symptoms may be particularly difficult. On the other hand, some of the samples were positive by the two techniques as long as three years after onset, thus confirming the long-term persistence of intrathecal antibody responses to HSV after acute encephalitis (Vandvik et al., 1985).

Both techniques were negative in the only case of HSV type 2 encephalitis (patient 5 in Table II). The antigens used in both methods are however not specific for HSV type 1. The immunoblotting technique had already detected anti-HSV intrathecal responses in cases of menigitis and encephalitis due to HSV type 2 (Boucquey et al., 1990; Monteyne et al., 1996). The same is true for ELISAs (Ashley et al., 1991). The results for patient n° 5 with HSE could, therefore, be considered as a false negative results for both methods. It should also be mentioned that this patient had the most benign form of encephalitis observed in this study, with a very rapid recovery. It is possible that the humoral response was weaker due to a low viral load. We cannot however exclude the possibility that our techniques are less sensitive for the detection of type 2 antibodies.

With the immunoblotting technique, we found a simultaneous antibody reactivity against HSV and VZV in some HSE, MS and OIND cases, while a VZV AI has not been systematically determined. In MS, the intrathecal synthesis of antibodies against various neurotropic viruses is well known (Sindic et al., 1994) and thus could include reactivity against both VZV and HSV. However, serological cross-reactions between HSV and VZV have been described with ELISA techniques (Ndumbe and Levinsky, 1985; Vandvik et al., 1985; Kühn et al., 1990) and may vary with the types and strains of viral antigens used. This cross-reactivity could also explain our positive results on immunoblots in some OIND cases, as in zoster meningitis (Fig. 2c). In such cases, the possibility of dual infections by both viruses is not excluded (Casas et al., 1996) but its demonstration requires the use of specific methods to detect either both genomes or antibody responses to specific epitopes.

Two MS cases were positive by one of the formulae, but negative by immunoblotting (patients MS 2 and 6 in Table III). In these cases, immunoblotting revealed a mirror pattern with faint bands present in both serum and CSF. It is therefore possible that, due to the difficult distinction between an intrathecal production of antibodies and a systemic production with passage through the barrier, false positive results were found in these cases by the formulae. On the other hand, when a strong reaction is present in both serum and CSF (indicated as +++/+++ in our semi-quantitative analysis of immunoblotting), some bands restricted to the CSF could be ignored. To increase the sensitivity in such cases, the samples should be further diluted and retested by immunoblotting.

While the four formulae used gave acceptable results in patients with a monospecific intrathecal response, as in HSE, differences appeared in cases of chronic inflammatory CNS disease such as MS. Only Reiber's formula takes into account a possible polyspecific intrathecal IgG synthesis; this is at variance with Ukkonen's formula which uses only Q_{IgG} and ignores Q_{lim}. In Stiernstedt's and Klapper's formulae, the use of Q_{Alb}

TABLE IV. Correlation Between AI Calculation and Antigen-Mediated Immunoblots (all samples)

	Immunoblots		
	−	+	Total
AI			
−	33	6	39
+	2	26	28
Total	35	32	67

$X^2 = 39.2$; $P < 0.005$.

as denominator may be disadvantageous, especially in early HSE samples when $Q_{IgG} < Q_{Alb}$.

Differences between the formulae and the immunoblotting were also most apparent when considering the polyspecific intrathecal antibody response in MS. Some discrepancies may be due to differences in the relative avidity of serum and CSF antibody. This can be investigated by performing end-point titration of serum and CSF, and comparing the slopes of the respective titrations. Such problems may also be clarified by performing the ELISA, as well as immunoblotting, in the presence of chaotropic agents (Luxton et al., 1990, 1995).

In the NIND group, a case of sylvian stroke was positive for intrathecal synthesis of anti-HSV antibodies as detected by both techniques, while the PCR procedure was negative. This could correspond to a humoral immune reaction due to exposure to a (latent) HSV antigen as a result of necrosis of the brain. HSV genome has been demonstrated, presumably in the latent form, in the brain of both animal models (Lynas et al., 1993), and human CNS tissue from individuals dying from nonneurological diseases (Baringer and Pisani, 1994). The positive results in the patient with sylvian stroke show also that detection of intrathecal HSV antibodies, by either method, cannot prove by itself that HSV is the etiological cause of the patient's condition.

In the present study, we did not evaluate other techniques that can be used to detect the intrathecal synthesis of antibodies, particularly methods in which the serum/CSF IgG or Albumin ratios do not have to be calculated. For example, antibody-capture ELISA techniques have been successfully applied (van Loon et al., 1989 and 1992). With that method, the proportion of specific antibodies in serum or CSF determines the magnitude of the ELISA signal. When the ELISA signal in the CSF exceeds that in the serum, it means that the proportion of specific HSV antibodies in CSF is higher than in serum and that intrathecal synthesis has occurred. The use of several antigens on one microtiter plate and of absorbance ratios to compare the specific IgG levels represents another variant allowing the demonstration of abnormalities of the blood-CSF barrier and the detection of intrathecal IgG synthesis (Mathiesen et al., 1988). These techniques require further evaluation, and comparison with the two methods used in the present study.

Nevertheless, both AI calculation (according to Reiber) and the immunoblotting technique were sensitive enough to be proposed as a routine method to confirm the diagnosis of HSE. The present study shows a good overall correlation between these two techniques ($X^2 = 39.2$; $P < 0.005$; Table IV). The choice of procedure in individual laboratories is therefore dependent upon available practical expertise.

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APPENDIX

Other members of the European Union Concerted Action for Virus Meningitis and Encephalitis are as follows: Paola Cinque, Division di Malattie Infettive, Ospedale San Raffaele, Milan, Italy; José M. Echevarria, C.N.M.V.I.S., Instituti de Salud Carlos III, Madrid, Spain; Bent Faber Vestergaard, Department of Virology, Statens Seruminstitut, Copenhagen, Denmark; Marianne Forsgren, Clinical Virology F68, Huddinge University Hospital, Huddinge, Sweden; Giuseppe Gerna, Institute of Infectious Diseases, University of Pavia, Pavia, Italy; Monica Grandien, Swedish Institute for Infectious Disease Control, Stockholm, Sweden; Tapani Hovi, Enterovirus Laboratory, National Public Health Institute, Helsinki, Finland; Paul Klapper, Manchester Central Laboratory Services, Manchester Royal Infirmary, Manchester, UK; Marjaleena Koskiniemi, Department of Virology, University of Helsinki, Helsinki, Finland; Pierre Lebon, Service de Bacteriologie, Virologie et Hygiene, Hôpital Saint Vincent de Paul, Paris, France; Annika Linde, Swedish Institute for Infectious Disease Control, Stockholm, Sweden; Peter Muir, Department of Virology, United Medical and Dental Schools of Guy's and St. Thomas's Hospitals, London, United Kingdom; Elisabeth Puchhammer-Stockl, Institute of Virology, University of Vienna, Vienna, Austria; Clive Taylor, Department of Virology, Newcastle General Hospital, Newcastle-upon-Tyne, UK, Volker ter Meulen, Institut für Virologie und Immunobiologie, Universitat Wurzburg, Wurzburg, Germany; Anton van Loon, Department of Virology, Academic Hospital, Utrecht, The Netherlands; Thomas Weber, Department of Neurology, Marienkrankenhaus Hamburg, Hamburg, Germany.